Inhibitory Effects of Naturally Occurring Compounds on Aflatoxin B_1 Biotransformation

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Effects of naturally occurring compounds from plants on biotransformation of a mycotoxin, aflatoxin B_1, were evaluated. Among 77 naturally occurring compounds tested, anthraquinones, coumarins, and flavone-type flavonoids were shown to be potent inhibitors of aflatoxin B_1-8,9-epoxide formation. Addition of the flavonoids galangin, rhamnetin, and flavone strongly inhibited mouse liver microsomal conversion of aflatoxin B_1 to aflatoxin B_1-8,9-epoxide, a metabolically activated mutagenic product. In contrast to these results, addition of isoflavonoids, catechins, terpenes, alkaloids, and quinones to mouse liver microsomes did not inhibit formation of aflatoxin B_1-8,9-epoxide. Formation of the aflatoxin B_1 reductase product, aflatoxicol, by chicken liver cytosols was strongly inhibited by curcumin, the diferuloylmethane present in turmeric and other *Curcuma* species. Curcumin analogues also showed inhibitory effects, and a structure−activity study established that β-diketone groups linked with two benzyl moieties were essential for inhibition of aflatoxicol formation. An additional 37 naturally occurring compounds tested did not inhibit formation of aflatoxicol. These results demonstrate that dietary constituents in certain fruits, vegetables, and spices may have significant inhibitory effects on metabolic transformation of aflatoxins to their hepatotoxic or carcinogenic derivatives or, alternatively, may promote their transformation into nontoxic products.

Keywords: *Aflatoxin B_1; aflatoxin B_1-8,9-epoxide; cytochrome P450; flavones; galangin; aflatoxicol; aflatoxin B_1 reductase; curcumin*

INTRODUCTION

Numerous epidemiological studies have shown that dietary components present in fruits and vegetables protect against certain types of cancer (*1*). A lower rate of tumor production has been found in some animals fed crude diets composed of various types of natural constituents (*2*−*4*). One of the mechanisms by which these compounds may exert putative anticancer effects is through interaction with cytochrome P450 enzymes in the liver to reduce activation of procarcinogen substrates to carcinogens (*5*).

Recently, attention has been focused on the inhibitory roles of natural constituents in suppressing the carcinogenic and mutagenic effects of aflatoxins (*6*−*8*). Aflatoxins are biologically active, secondary metabolites produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus* (*9*). Aflatoxin B_1 (**1**; Figure 1) and structurally related difuranocoumarin compounds are a major concern to public health, mainly for their potential as powerful hepatotoxins and carcinogens to humans and their proven toxicity to animals, birds, and fish (*10*, *11*). The mechanism through which aflatoxins are genotoxic appears to result from formation of a single initial DNA adduct by the oxidized form, aflatoxin B_1-8,9-epoxide (**2**; Figure 1) with the guanyl N_7 atom (*12*−*14*). Therefore, the consumption of foodstuffs contaminated by aflatoxins may cause acute and chronic hepatotoxicity, leading to hepatocarcinogenesis and mutagenesis. This is of special concern in developing countries where endemic levels of the hepatitis B virus exacerbate the public health risk from exposure to aflatoxin (*15*).

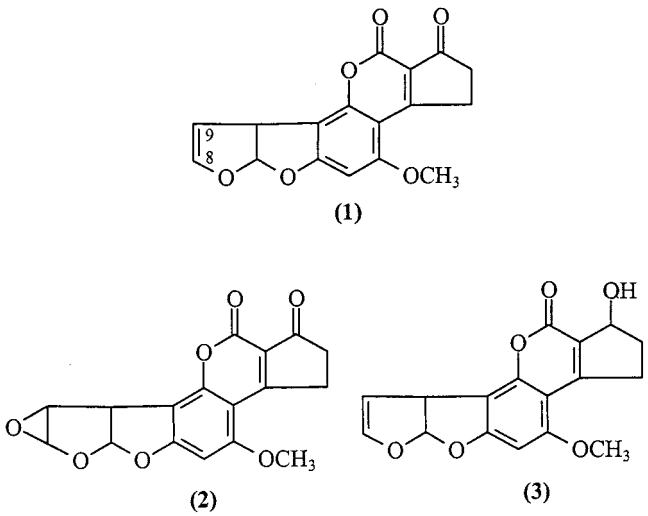

Figure 1. Structures of aflatoxin B_1 (**1**) and its main metabolites, aflatoxin B_1-8,9-epoxide (**2**) and aflatoxicol (**3**).

Toxic and carcinogenic effects of aflatoxins are intimately linked with their biotransformation into the corresponding 8,9-epoxides. The enzymes mainly involved in biotransformation of aflatoxin B_1 in animals, birds, and fishes are cytochromes P450 of the liver (*16*,

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17). An alternative biotransformation of aflatoxin B_1 to aflatoxicol (**3**; Figure 1) is mediated by a reductase, and the product can be reconverted to aflatoxin B_1 by a dehydrogenase. Aflatoxin B_1 reductase and aflatoxicol dehydrogenase are not well studied, but this mechanism is considered to be very important because aflatoxicol may play a role as a reservoir of aflatoxin B_1 in some organisms. Aflatoxin biotransformation may be influenced by phytochemicals in the diet that modulate various biological activities. For example, natural antioxidants could suppress formation of aflatoxin epoxides or prevent dehydrogenation of aflatoxicol retroactively to aflatoxin B_1. The objective of this study was therefore to investigate the effects of several classes of naturally occurring compounds, common in fruits, vegetables, or spices, on formation of the 8,9-epoxide derivative or aflatoxicol from aflatoxin B_1 and to elucidate structural features governing interaction between such natural products and cytochromes P450 or reductases. The information obtained was expected to provide a fundamental basis for further investigation of effects of specific dietary compounds in mitigating toxification or promoting detoxification of aflatoxins.

MATERIALS AND METHODS

Chemicals. Aflatoxins B_1 and G_1 were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Aflatoxin B_1-8,9-epoxide was synthesized as previously described (*18*). Aflatoxin epoxide−glutathione conjugate was biosynthesized according to the protocols of Raney et al. (*19*). All synthesized chemicals were pure as determined by HPLC and MS. *m*-Chloroperbenzoic acid and dithiothreitol (DTT) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Phenylmethanesulfonyl fluoride (PMSF) and reduced glutathione (GSH) were purchased from Sigma. All purchased chemicals were of the highest grade commercially available.

Biological Material. Mouse liver (wild C57BL6J) was kindly provided by Dr. Y. S. Moon, University of California, Berkeley, CA, and fresh chicken livers were purchased from a local butcher in Albany, CA.

Preparation of Animal Liver Homogenates. All preparations were carried out at 4 °C. One gram of mouse or chicken liver was homogenized in a glass homogenizer with 15 mL of 100 mM phosphate buffer, pH 7.4, containing 0.4 mM PMSF, 0.1 mM DTT, and 1 mM EDTA. The resultant homogenates were filtered through four layers of cheesecloth. The homogenates were centrifuged at 12100*g* at 4 °C for 20 min using an Eppendorf centrifuge 5417R. Supernatants were reserved as crude enzyme extracts.

Crude enzyme extracts were transferred to 15 mL polycarbonate ultracentrifuge tubes and centrifuged at 100000*g* at 4 °C for 1 h, including acceleration time, in a Beckman L8-M ultracentrifuge using a Ti 70 rotor. The supernatant was reserved as the cytosolic fraction. The microsomal pellet was rinsed twice with 4 mL of resuspension buffer, 200 mM phosphate buffer, pH 7.4, containing 1 mM EDTA, and fully resuspended in a glass homogenizer brought to a volume of 5 mL.

Inhibition of Aflatoxin B_1-8,9-epoxide Production from Aflatoxin B_1 by Mouse Liver Microsomal Proteins Using Naturally Occurring Compounds. To detect aflatoxin B_1-8,9-epoxide, mouse cytosolic glutathione *S*-transferase (GST) was employed to conjugate GSH to any aflatoxin epoxide produced by mouse liver preparations. In a typical experiment, 10 μL of a 1000 ppm of aflatoxin B_1 solution (in DMSO) was added to 0.6 mL of a reaction mixture consisting of 92 mM sodium phosphate buffer, pH 7.4, 0.5 mM GSH, 0.5 mM NADPH, and mouse liver cytosolic fraction (2.0 mg of protein mL^{-1}). Mouse liver microsomal fractions (1.5 mg of protein mL^{-1}) were added to the reaction mixture. After a preincubation period of 10 min at 37 °C, NADPH was added to the reaction mixture to initiate the reaction. After 3 h of incubation, the reaction was stopped by adding 1 mL of ice-cold methanol containing aflatoxin G_1 (10 μM) as an internal standard. This reaction mixture was centrifuged at 12100*g* for 10 min at room temperature. The supernatant was analyzed by a reversed-phase Supelcosil LC-18 column (250 × 4.6 mm) equipped with a fluorescence detector. The mobile phase was a mixture of water/acetonitrile/methanol (60:20:20).

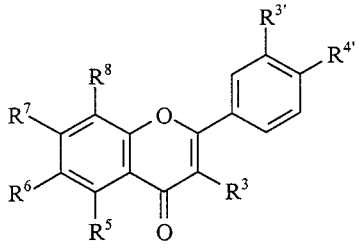
Inhibition of Aflatoxin B_1 Metabolism to Aflatoxicol by Chicken Liver Cytosol Using Naturally Occurring Compounds. Metabolism of aflatoxin B_1 to aflatoxicol was studied using an incubation mixture (250 μL final volume) consisting of 92 mM sodium phosphate buffer, pH 7.4, 0.5 mM NADPH, and 2 mg mL^{-1} protein of the chicken liver cytosol preparation. After a preincubation period of 10 min at 37 °C, aflatoxin B_1 (10 μL of a 1000 ppm solution in DMSO) was added as substrate to the reaction mixture. After 1 h of incubation, reactions were stopped by adding 1 mL of ice-cold methanol containing aflatoxin G_1 (10 μM) as an internal standard. This mixture was centrifuged at 12100*g* for 10 min at room temperature. The supernatant was analyzed by a reversed-phase Supelcosil LC-18 column (250 × 4.6 mm) equipped with a fluorescence detector. The mobile phase was a mixture of water/acetonitrile/methanol (60:20:20).

Enzyme Kinetics. The IC_{50} (expressed as micromolar) is the concentration at which 50% inhibition of aflatoxin B_1-8,9-epoxide or aflatoxicol formation was reached as calculated from the dose−response curve. The kinetics of aflatoxin B_1 reductase were evaluated using different concentrations of the substrate aflatoxin B_1 in the absence as well as in the presence of a constant concentration of curcumin. The kinetic parameters (K_m and V_{max}) were calculated using the Lineweaver−Burk plot, and the type of inhibition was inferred from the changes produced by curcumin on the values of K_m and V_{max}. The K_i value of curcumin was calculated by using the equation $K_{m,app} = K_m[1 + ([I]/K_i)]$.

RESULTS

The effects of flavonoids on aflatoxin B_1-8,9-epoxide formation in mouse liver are shown in Table 1. Among the 34 flavonoids tested (Table 1), the flavone type (Figure 2) generally exhibited potent inhibitory effects on aflatoxin epoxide formation. Galangin (**7**) (IC_{50} = 1.19 μM) and rhamnetin (**10**) (IC_{50} = 1.29 μM) were most potent, followed by the parent compound, flavone (IC_{50} = 1.32 μM). Naringenin (**13**; 5,7,4′-trihydroxyflavanone) (IC_{50} = 12.0 μM) showed strong inhibition of epoxide formation, whereas the other flavanones tested did not show activity. Chalcone (IC_{50} = 14.3 μM) had a 10.8-fold lower inhibitory activity than flavone. Flavonoids with a C2−C3 double bond were more effective than the corresponding saturated homologues, as shown by comparison of apigenin (**5**; 5,7,4′-trihydroxyflavone) (IC_{50} = 9.52 μM) with naringenin (**13**) (IC_{50} = 12.0 μM). With the exception of galangin versus acacetin (**4**), flavonoids with methylated hydroxyl groups, such as rhamnetin and tangeretin (**11**), were more effective inhibitors than those having free hydroxyl groups, such as quercetin. In general, for polyhydroxylated flavonoids, an increasing number of hydroxyl groups decreased the effectiveness, as indicated by comparison of the series chrysin (**6**), kaempferol (**8**), and quercetin (**9**). Glycosylated flavonoids lacked inhibitory effects on aflatoxin epoxide formation, as exemplified by comparison of naringin (naringenin 7-rhamnoglucoside) and prunin (naringenin 7-glucoside) versus naringenin. The isoflavone pratol (**14**) showed reasonable activity, but most anthocyanidins, isoflavonoids, and catechins tested did not have any inhibitory effect on epoxide formation.

Coumarins (Table 2) showed strong inhibitory effects, and angelicin (IC_{50} = 1.83 μM) was the most potent



Flavone Class

(4) Acacetin: $R^5 = R^7 = -OH$, $R^{4'} = -OMe$

(5) Apigenin: $R^5 = R^7 = R^{4'} = -OH$

(6) Chrysin: $R^5 = R^7 = -OH$

(7) Galangin: $R^3 = R^5 = R^7 = -OH$

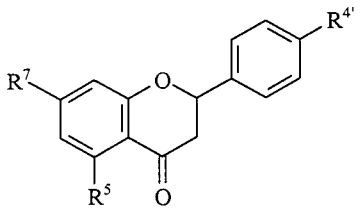
(8) Kaempferol: $R^3 = R^5 = R^7 = R^{4'} = -OH$

(9) Quercetin: $R^3 = R^5 = R^7 = R^{3'} = R^{4'} = -OH$

(10) Rhamnetin: $R^3 = R^5 = R^{3'} = R^{4'} = -OH$, $R^7 = -OMe$

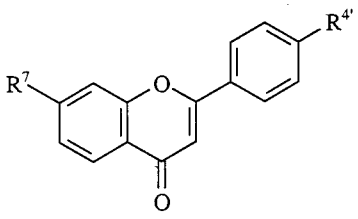
(11) Tangeretin: $R^5 = R^6 = R^7 = R^8 = R^{4'} = -OMe$

(12) Tectochrysin: $R^5 = -OH$, $R^7 = -OMe$



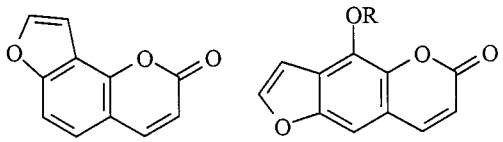
Flavanone Class

(13) Naringenin: $R^5 = R^7 = R^{4'} = -OH$



Isoflavone Class

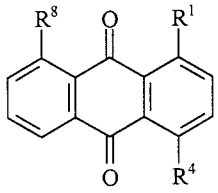
(14) Pratol: $R^7 = -OH$, $R^{4'} = -OMe$



Furocoumarin Class

(15) Angelicin (16) Imperatorin: $R = -CH_2CH{=}CMe_2$

(17) Xanthotoxin: $R = -Me$



Anthraquinone Class

(18) 1,4-Dihydroxyanthraquinone: $R^1 = R^4 = -OH$

(19) 1,8-Dihydroxyanthraquinone: $R^1 = R^8 = -OH$

Figure 2. Structures of naturally occurring compounds tested showing highest activity levels for the inhibition of aflatoxin B_1-8,9-epoxide formation; R = −H when not specified.

among the coumarins tested in inhibiting aflatoxin B_1-8,9-epoxide formation. From the limited number of compounds tested, furanocoumarins (Figure 2), including imperatorin (**16**) and xanthotoxin (**17**), appeared to be somewhat better inhibitors than simpler coumarins. Anthraquinones were also significant inhibitors, and 1,4-dihydroxyanthraquinone (**18**) (IC_{50} = 4.37 μM) was a slightly better inhibitor than 1,8-dihydroxyanthraquinone (**19**) (IC_{50} = 5.81 μM), with 1,8-dihydroxy-3-methylanthraquinone (IC_{50} = 43.7 μM) showing less inhibitory activity. Naphthoquinone and its derivatives, 2-methylnaphthoquinone, juglone, and plumbagin, which have been shown to affect aflatoxin production in *A. flavus* (*20*), were insufficiently soluble in the reaction solution to permit their evaluation.

In general, those terpenes tested were not potent inhibitors of aflatoxin B_1-8,9-epoxide formation. Inhibitory activities of monoterpenes varied with structural differences. Three hydroxylated monoterpenes, anethole (IC_{50} = 265 μM), carvacrol (IC_{50} = 415 μM), and eugenol (IC_{50} = 182 μM), showed moderate inhibitory activity and exhibited higher activity than nonhydroxylated monoterpenes such as *trans*-cinnamaldehyde, estragole, and fenchone. A hydroxylated tropolone, hinokitiol (IC_{50} = 27.5 μM), had a stronger inhibitory effect on epoxide formation than tropolone, which had no effect. Three citrus limonoids, limonin, nomilin, and obacunon, and the structurally related terpenoid, gedunin, did not show any inhibitory effects. The one capsaicinoid tested, capsanthin, also did not show any inhibition. The alkaloids tested, which included berberine, bicuculine, cinchonidine, cinchoinine, palmitine, pipereicosalidine, piperlongumine, piperoctadecalidine, piperettine, quinidine, and quinine, belonging to several different structural classes, did not inhibit aflatoxin epoxide formation. Only piperine (IC_{50} = 740 μM) had very weak inhibitory

Table 1. IC_{50} Values for Inhibition of Biotransformation of Aflatoxin B_1 to Aflatoxin B_1-8,9-epoxide by Flavonoids Using Mouse Liver Microsomal Cytochrome P450

compound[a]	structure type	IC_{50} (ppm)	IC_{50} (μM)
acacetin (**4**)	flavone	2.06	7.25
angolensin	isoflavone	>500	
apigenin (**5**)	flavone	2.57	9.52
(+)-catechin	catechin	>500	
(−)-catechin	catechin	>500	
chalcone	flavanone	2.97	14.3
chrysin (**6**)	flavone	2.08	8.19
cyanidin	anthocyanin	>500	
daidzein	isoflavone	>500	
diosmin	flavone	>500	
(+)-epicatechin	catechin	>500	
(−)-epicatechin	catechin	>500	
fisetin	flavone	88.1	310
flavone	flavone	0.48	1.31
galangin (**7**)	flavone	0.32	1.19
genistein	isoflavone	>500	
genistin	isoflavone	>500	
hesperidin	flavanone	>500	
7-hydroxyflavone	flavone	1.30	5.46
isosakuranetin	flavanone	>500	
kaempferol (**8**)	flavone	3.28	8.73
malvidin	anthocyanin	>500	
morin	flavone	53.1	175
myricetin	flavone	>500	
naringenin (**13**)	flavanone	3.37	12.0
naringin	flavanone	>500	
neohesperidin	flavanone	>500	
peonidin	anthocyanin	>500	
pratol (**14**)	flavone	3.23	12.0
prunin	flavanone	>500	
quercetin (**9**)	flavone	7.55	24.8
rhamnetin (**10**)	flavone	0.41	1.29
tangeretin (**11**)	flavone	6.48	17.4
tectochrysin (**12**)	flavone	1.71	6.37

[a] Bold-face numbers in parentheses correspond to numbered structures in Figure 2.

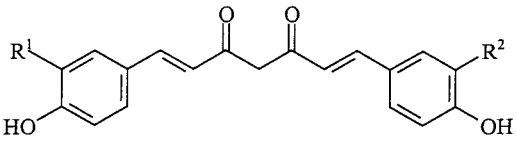
Table 2. IC_{50} Values for Inhibition of Biotransformation of Aflatoxin B_1 to Aflatoxin B_1-8,9-epoxide by Coumarins and Quinones Using Mouse Liver Microsomal Cytochrome P450

compound[a]	structure type	IC_{50} (ppm)	IC_{50} (μM)
alizarin-3-methylimino-diacetic acid	anthraquinone	54.1	140
angelicin (**15**)	furanocoumarin	0.34	1.83
1,4-dihydroxyanthraquinone (**18**)	anthraquinone	1.05	4.37
1,8-dihydroxyanthraquinone (**19**)	anthraquinone	1.39	5.81
1,8-dihydroxy-3-methylanthra-quinone	anthraquinone	11.2	43.7
imperatorin (**16**)	furanocoumarin	0.72	2.66
nordalbergin	coumarin	3.27	12.8
purpurin	anthraquinone	3.82	14.9
scopoletin	coumarin	11.4	64.9
xanthotoxin (**17**)	furanocoumarin	0.54	2.50

[a] Bold-face numbers in parentheses correspond to numbered structures in Figure 2.

properties. Curcumin, methyl gallate, nordihydroguaiaretic acid, and trimethoprim did not reveal any effect on the epoxide formation in mouse liver.

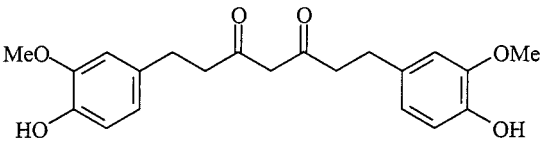
To determine inhibitory effects on aflatoxicol (**3**) formation by reductase, 38 naturally occurring compounds were tested. Curcumin (**20**) was a potent inhibitor of aflatoxicol formation (IC_{50} = 81.0 μM), and a series of derivatives of curcumin were also tested (Figure 3). The inhibitory mode of action by curcumin on aflatoxin B_1 reductase was determined to be competitive when analyzed by a Lineweaver−Burk plot, with the curves obtained from the uninhibited enzyme and from the one



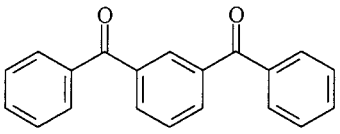
(20) Curcumin: $R^1 = R^2 = -OMe$

(21) Demethoxycurcumin: $R^1 = -OMe$, $R^2 = -H$

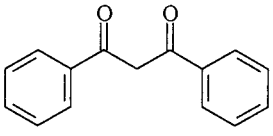
(22) Bisdemethoxycurcumin: $R^1 = R^2 = -H$



(23) Tetrahydrocurcumin



(24) 1,3-Dibenzoylbenzene



(25) Dibenzoylmethane

Figure 3. Structures of curcumin and analogues tested for inhibition of aflatoxin B_1 reductase.

Table 3. IC_{50} Values for Inhibition of Biotransformation of Aflatoxin B_1 to Aflatoxicol by Curcumin and Its Derivatives Using Chicken Liver Cytosols

compound[a]	IC_{50} (μM)
curcumin (**20**)	81
demethoxycurcumin (**21**)	75
bisdemethoxycurcumin (**22**)	103
tetrahydrocurcumin (**23**)	43
1,3-dibenzoylbenzene (**24**)	129
dibenzoylmethane (**25**)	270
p-hydroxy-3-methyl-*trans*-cinnamic acid	>500
2,4-pentanedione	>500
benzophenone	>500

[a] Bold-face numbers in parentheses correspond to numbered structures in Figure 3.

concentration of curcumin intersecting on the vertical axis. The K_i value of curcumin on aflatoxin B_1 reductase was 0.12 mM. Tetrahydrocurcumin (**23**) (IC_{50} = 43.0 μM) and demethoxycurcumin (**21**) (IC_{50} = 75.0 μM) were stronger inhibitors than curcumin, whereas bisdemethoxycurcumin (**22**) (IC_{50} = 103 μM) showed less inhibition of aflatoxicol formation (Table 3). The synthetic analogues 1,3-dibenzoylbenzene (**24**) and dibenzoylmethane (**25**) possessed some inhibitory effects, but significantly less than curcumin. Benzophenone and 2,4-pentanedione were not inhibitors, and *p*-hydroxy-3-methyl-*trans*-cinnamic acid, a biosynthetic precusor of curcumin, was also not an inhibitor of aflatoxicol formation. Berberine, kaempferol, and quercetin had similar IC_{50} values of 50 mg/L. There was no detectable inhibitory effect (>50 mg/L) in the other tested compounds, encompassing several structural types, which included acacetin, alizarin-3-methyliminodiacetic acid, anethole, angolensin, carvacrol, (+)-catechin, (−)-catechin, chrysin, daidzein, dicoumarol, 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone, 1,8-dihydroxy-3-methylanthraquinone, (+)-epicatechin, (−)-epicatechin, estragole, eugenol, fenchone, fisetin, genistein, guaiacol, 4-hydroxycoumarin, 7-hydroxyflavone, imperatorin, kaempferol, methyl gallate, morin, nordalbergin, nordihydroguaiaretic acid, pratol, purpurin, quercetin, scopoletin, and umbelliferone.

DISCUSSION

A comparison of the biochemical effects of flavones, coumarins, and quinones revealed a strong inhibitory effect on aflatoxin B_1 transformation to aflatoxin B_1-8,9-epoxide by mouse liver microsomes. In contrast, isoflavones, flavanones, flavone glycosides, monoterpenes, curcuminoids, limonoids, and alkaloids were less active. Flavanoids are common natural constituents of the foliage and fruit of many edible higher plants (*21*). Inactivation of aflatoxin B_1-8,9-epoxide formation by flavones or other natural products has not previously been investigated using mouse liver microsomes. However, the results of another study (*22*) demonstrated that some naturally occurring flavones, including flavone, tangeretin, and nobiletin, as well as 7,8-benzoflavone, stimulated metabolism of aflatoxin B_1 to mutagens and to aflatoxin B_1-2,3-dihydrodiol by human liver microsomes. It is likely that different structural features may be required for inhibition of human or mouse liver microsomal monooxygenases. Biotransformation of aflatoxin B_1 is closely linked with toxic and carcinogenic effects through production of the 8,9-epoxide. Cytochrome P450 enzymes (Cyt P450) are primarily involved in biotransformation of aflatoxin B_1, and it has been reported that at least five P450s (1A2, 2A3, 2B7, 3A4, and 3A5) can convert aflatoxin B_1 into the 8,9-epoxide in humans (*16*). Among these, cytochrome P450 3A4 is generally agreed to be the most abundant cytochrome P450 enzyme present in both the liver and small intestine and to play the most important role in formation of the epoxide (*23*, *24*). However, cytochrome P450 2A5, initially identified as testosterone 15α-hydroxylase type II and now more specifically known as coumarin 7-hydroxylase, is also involved in metabolic activation of aflatoxin B_1 in the mouse (*17*). Interestingly, the results of Tsyrlov et al. (*5*) showed that CYP 1A1 and CYP 1A2 differed in their sensitivities to hydroxylated and nonhydroxylated flavonoids, because α-naphthoflavone and flavone did not change the benzo[*a*]pyrene 3-hydroxylation activity of human CYP 1A2 but inhibited its 7-methoxyresorufin *O*-dealkylation activities in mouse and human CYP 1A2. In contrast, hydroxylated flavonoids increased 7-methoxyresorufin *O*-dealkylation activities. Thus, the difference in sensitivity between mouse and human CYP 1A2 to flavonoids has not been determined. Therefore, activation of aflatoxin B_1 epoxidation by human liver microsomes with some flavones may result from different components of cytochromes P450 than those of mouse liver microsomes.

Galangin (**7**) was a potent inhibitor of aflatoxin B_1-8,9-epoxide formation by mouse liver microsomes. Galangin is a member of the flavonol class of flavonoids and is present in high concentrations in *Alpinia officinarum*, which has been used as a spice and as a herbal medicine for a variety of ailments in Asia for centuries. Galangin has been shown to inhibit proliferation of breast cancer tumor cells (*25*), to prevent genotoxicity of *N*-methyl-*N*-nitrosourea (*26*), and to inhibit cytochrome P450 hydroxylase activity in human liver microsomes (*22*, *27*). However, galangin has been suggested to be a substrate of certain cytochromes P450 which, through hydroxylation of the B ring, metabolize it to other products. Silva et al. (*28*) showed that galangin was sequentially transformed to kaempferol (**8**) and then to quercetin (**10**) by a mechanism dependent on cytochrome P450 reactions. Certain metabolites

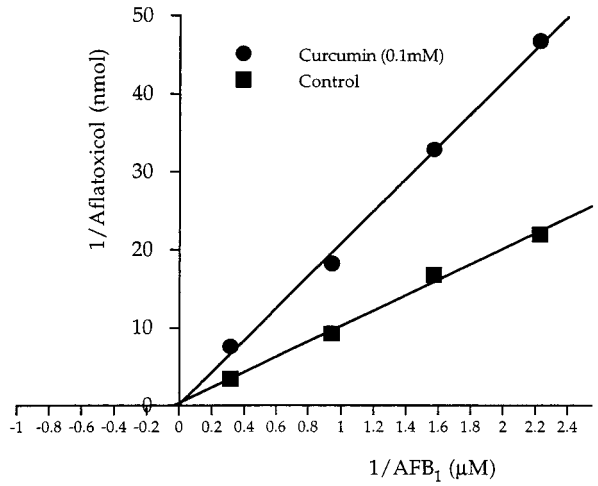


Figure 4. Lineweaver−Burk plot of inhibition by curcumin of aflatoxin B_1 reductase, forming aflatoxicol from aflatoxin B_1.

of galangin are indicated to be mutagenic by the *Salmonella typhimurium* reversion assay and can induce chromosomal aberrations in V79 cells. Use of galangin as a chemopreventive drug for cancer should therefore only be considered with extreme caution. Other active inhibitors of aflatoxin B_1-8,9-epoxide formation, such as acacetin (**4**), apigenin (**5**), chrysin (**6**), flavone, 7-hydroxyflavone, kaempferol (**8**), and rhamnetin (**10**), have also been shown to be mutagenic in vitro in the *S. typhimurium* assay (*29*).

Furanocoumarins, also referred to as furocoumarins, are found in the plant families Umbelliferae and Rutaceae. Some furanocoumarins have been used in the cure of vitiligo and for therapeutic treatment of psoriasis (*30*). A number of linear furanocoumarins have been found to inhibit activities of drug-metabolizing enzymes, possibly infuenced by the presence of a prenyl side chain (*31*). Shin and Woo (*32*) demonstrated that the inhibitory potencies of angelicin and psoralen toward rat liver microsomal aminopyridine *N*-demethylase and hexobarbital hydroxylase activities were significantly weaker than those of other furanocoumarins possessing side-chain moieties, including imperatorin, isoimperatorin, oxypeucedanin, and isooxypeucedanin. However, our findings indicate that furanocoumarins having no side chain are stronger inhibitors of aflatoxin B_1-8,9-epoxide formation than furanocoumarins having a prenyl side chain. In addition, our results show that longer prenyl side chains on furanocoumarins produce progressively lower inhibition of epoxidation activity.

Anthraquinones are found in higher plants and fungi and have been widely used as colorants in foods, cosmetics, hair dyes, and textiles and as phytotherapeutic drugs (*33*, *34*). Anthraquinones have been characterized as mutagens with genotoxicity caused by their ability to intercalate into DNA because of their planar structures (*35*, *36*). 1,4-Dihydroxyanthraquinone (**18**) and 1,8-dihydroxyanthraquinone (**19**) have the potential to inhibit cytochrome P450 1A1/2 ethoxyresorufin-*O*-deethylase activity (*37*, *38*), and these same compounds strongly inhibited the formation of aflatoxin B_1-8,9-epoxide in mouse liver microsomes.

Curcumin (**20**) (Figure 3) was found to competitively inhibit chicken liver cytosolic aflatoxin B_1 reductase biotransformation of aflatoxin B_1 into aflatoxicol (Figure 4). Curcumin is a β-diketone constituent of the spice

turmeric, *Curcuma longa* L., and has been found to possess anticarcinogenic properties in several model systems (*39*, *40*). Recently, curcumin was considered for further evaluation as a candidate chemopreventive agent (*41*). When we tested a series of analogues of curcumin (Figure 3) to determine the structural features necessary for their inhibitory effects on aflatoxicol formation, tetrahydrocurcumin (**23**) was a significantly stronger inhibitor than curcumin, whereas demethoxycurcumin (**21**), lacking a methoxy group on one of the aromatic rings, possessed only a slightly stronger inhibitory effect. However, bisdemethoxycurcumin (**22**), which lacks methoxy groups on both of the aromatic rings, showed less inhibitory activity than curcumin. Dibenzoylmethane (**25**) is similar in structure to curcumin in possessing a β-diketone moiety linking two phenyl groups but without the intermediate double bonds. This compound also possessed inhibitory activity on aflatoxicol formation, but 3-fold less than curcumin. 1,3-Dibenzoylbenzene (**24**), which is analogous to dibenzoylmethane but with the central methylene group replaced by a benzene ring, showed about a 50% lower inhibitory effect on aflatoxicol formation than did curcumin. However, 2,4-pentanedione, a simple β-diketone lacking terminal phenyl groups, did not have any inhibitory effect. Benzophenone, which possesses a single ketone group linking two phenyl groups, also did not show any inhibitory activity. Therefore, among the curcumin analogues, a β-diketone unit linking two phenyl groups is an essential structural feature for inhibition of aflatoxin B_1 reductase. Substitution of a benzyl group for a phenyl group enhances inhibition of aflatoxicol formation, and the presence of hydroxyl or methoxyl substituents appears to further increase inhibitory activity.

The results of this study show that flavones, coumarins, and anthraquinones have a significant influence on inhibition of aflatoxin B_1 biotransformation to aflatoxin B_1-8,9-epoxide by cytochrome P450 enzymes of mouse liver. However, anthocyanidins and catechins do not inhibit epoxide formation, and selected terpenoids and alkaloids also do not possess any inhibitory effect. Curcuminoids and structural analogues are potent inhibitors of aflatoxicol formation by chicken liver reductases. Their β-diketone moieties linking two phenyl groups are essential for this inhibitory effect. These findings provide a basis for further study on relationships between naturally occurring compounds in the diet and reduced risk of aflatoxin-induced carcinogenesis in vivo. From the range of chemical structural types examined in this study, specific compounds having potent inhibitory activity, together with known occurrence in foodstuffs commonly consumed, could be selected for more intensive investigation of their potential effects on human hepatic metabolism of aflatoxins.

SAFETY

Aflatoxins are hazardous due to their potential hepatotoxicity and carcinogenicity, and aflatoxin epoxides are potent mutagenic agents. As solids they are electrostatic and should be handled using appropriate containment procedures and respiratory masks to prevent inhalation. The use of gloves and well-ventilated fume hoods and careful destruction of residues with NaOCl are essential for manipulation of these compounds.

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